Probiotic supplementation reduces systemic inflammation in dialysis patients: the effect on circulating regulatory T-cells and pro-inflammatory monocytes

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Research

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Abstract

Background

Emerging evidence suggests that intestinal dysbiosis contributes to systemic inflammation and cardiovascular diseases in dialysis patients. The purpose of this study was to evaluate the effects of probiotic supplementation on various inflammatory parameters in hemodialysis (HD) patients.

Methods

Twenty-two patients with maintenance HD were enrolled (Institutional Review Board No. 2018AN0346). These patients were treated twice a day with $2.0 \times 10^{10}$ colony forming units of a combination of *Bifidobacterium bifidum* BGN4 and *Bifidobacterium longum* BORI for 3 months. The microbiome and fecal short chain fatty acids (SCFAs) were analyzed. The percentages of CD14$^+$ CD16$^+$ pro-inflammatory monocytes, and CD4$^+$ CD25$^+$ regulatory T-cells (Tregs) before and after probiotic supplementation were determined by flow cytometry. Serum levels of calprotectin and cytokine responses upon lipopolysaccharide (LPS) challenge were compared before and after probiotic supplementation.

Results

Fecal SCFAs increased significantly after probiotic supplementation. Serum levels of calprotectin and IL-6 upon LPS stimulation significantly decreased. The anti-inflammatory effects of probiotics were associated with a significant increase in the percentage of CD4$^+$ CD25$^+$ Tregs (3.5% vs 8.6%, p < 0.05) and also with a decrease of CD14$^+$ CD16$^+$ pro-inflammatory monocytes (310 vs. 194/mm$^2$, p < 0.05).

Conclusion

Probiotic supplementation reduced systemic inflammatory responses in HD patients and this effect was associated with an increase in Tregs and a decrease in pro-inflammatory monocytes. Hence, targeting intestinal dysbiosis might be a novel strategy for decreasing inflammation and cardiovascular risks in HD patients.

Trial registration

The study was retrospectively registered in Clinical Research Information Service (CRIS) (KCT0005417) (09/09/2020).

Introduction

Maladaptive and persistent inflammation has been recognized as an important player in the development of cardiovascular diseases and also as a predictor of mortality in patients with chronic kidney disease (CKD)[1–4]. Although not completely understood, retention of uremic solutes, oxidative stress, immune dysfunction, or dialysis-related factors including repeated exposure to dialysis membranes, contaminated dialysis water, and recently, intestinal dysbiosis, have been implicated as important culprits in the development of chronic inflammation.

Significant alterations in the diversity and number of operational taxonomic units (OTUs) associated with increased intestinal permeability have been demonstrated in preclinical and clinical studies. Uremic milieu, slower
colonic transit time, bowel edema due to volume overload, restrictions of fiber-rich diets, medications including phosphate binders, and frequent use of antibiotics might be factors contributing to the generation and maintenance of dysbiosis [5–8].

Probiotics are “live microorganisms that, when administered in adequate amounts, confer a health benefit to the host” [9]. Probiotics have been shown to partially restore normal intestinal microbiota and reduce the level of uremic toxins and systemic inflammation. A recent study by Soleiman et al., showed improvement of glycemic control and reductions in C-reactive proteins (CRP) in diabetic patients undergoing HD [10]. One of the mechanisms of probiotic-induced anti-inflammatory effects might be mediated by probiotic-induced immune modulation.

CD4+ CD25+ regulatory T-cells (Tregs) are a subpopulation of T-cells with regulatory function and have been demonstrated to be effective in reducing inflammation. CD14+ CD16+ nonclassical monocytes are a subset of pro-inflammatory monocytes which increase in advanced CKD patients. Lee et al., previously demonstrated a positive correlation between these cells and vascular stiffness, suggesting the possible important role of this monocyte subset in increased cardiovascular risks in CKD patients [11].

The purpose of this study was to assess the effect of probiotic supplementation on chronic inflammation in maintenance HD patients. We analyzed the microbiome, fecal short chain fatty acids (SCFAs), and inflammatory responses before and 3 months after probiotic supplementation. The percentage of circulating Tregs and the number of CD14+ CD16+ monocytes were also measured by flow cytometry. The effect of probiotic supplementation on these parameters was also examined 4 months after the discontinuation of probiotics.

**Material And Methods**

**Study design**

Enrolled patients included those aged ≥ 18 years undergoing maintenance dialysis for more than 3 months at Korea University Anam Hospital, a tertiary hospital in Seoul, South Korea, from Nov to Dec 2018. Exclusion criteria were patients who: (a) were on HD for acute kidney injury; (b) were on HD less than twice per week; (c) had uncontrolled diarrhea or gastrointestinal infection; (d) were treated with oral or intravenous antibiotics within 4 weeks of enrollment; and (e) were actively being treated for cancer, or with immunosuppressive drugs. Among the 23 patients providing informed consent, 1 patient withdrew from the study and 22 patients completed the trial. These patients were treated with sachets [2 g mixtures of probiotics containing 7.0×10⁹ colony forming units (CFU)/g of *Bifidobacterium bifidum* BGN4 and 2.0×10⁹ CFU/g of *Bifidobacterium longum* BORI] twice per day for 3 months. *B. bifidum* BGN4 and *B. longum* BORI were isolated from the feces of healthy breast-fed infants, and have been used as probiotics since 2000 [12–16]. Its complete genomic sequence was reported to GenBank [17]. *B. longum* BORI has been shown to shorten the duration of diarrhea in a clinical study of infants infected with rotavirus [12]. A safety assessment of *B. bifidum* BGN4 and *B. longum* BORI concerning ammonia production, hemolysis of blood cells, biogenic amine production, antimicrobial susceptibility patterns, antibiotic resistance gene transferability, polymerase chain reaction (PCR) data on antibiotic resistance genes, mucin degradation, genome stability, and the presence of virulence factors has been recently reported [18] Both strains have been considered Generally Recognized As Safe by the United States Food and Drug Administration (US FDA) (GRN813 for *B. longum* BORI and GRN814 for *B. bifidum* BGN4; https://www.accessdata.fda.gov/scripts/fdcc/?)
Blood and fecal samples were obtained at baseline and at 3 and 6 months. The study protocol was approved by the Korea University Medical Center Institutional Review Board (IRB No. 2018AN0346) Written informed consent was provided by all participants.

**Laboratory measurements**

Routine laboratory measurements including complete blood counts with white blood cell differentials, CRP, albumin, blood urea nitrogen, creatinine, and electrolytes were obtained before the dialysis session. Patients’ demographic factors and current medications were also recorded.

Blood and fecal samples were centrifuged at 2,500×g for 15 min and stored at -80°C for calprotectin measurements. Samples were analyzed using an enzyme-linked immunosorbent assay (ELISA - LSBio, LS-F9275, Seattle, WA, USA) according to the manufacturer's instructions.

**Flow cytometric detection of Tregs and pro-inflammatory monocyte subsets**

Blood samples were collected before the dialysis session. Whole blood (2.5 ml) was collected in a heparinized tube and 200 µl aliquots of heparinized blood were stained for 15 min at room temperature with either anti-human CD4 conjugated with allophycocyanin (CD4-APC), anti-human CD25 conjugated with phycoerythrin (CD25-PE) antibodies for detection of Tregs, anti-human CD14 conjugated with fluorescein isothiocyanate (CD14-FITC), or anti-human CD16 conjugated with allophycocyanin (CD16-APC) antibodies for the detection of monocytes (BD Biosciences, San Jose, CA, USA). After red blood cell lysis and washing, flow cytometric detection of Tregs and the CD14^+ CD16^+ pro-inflammatory monocyte subset from among 10^6 cells were performed (FACSCalibur™; BD Biosciences, San Jose, CA, USA) and analyzed by FlowJo v8.5.2. The percentage of cells or the actual number of cells at baseline were compared to the values 3 months after initiation of probiotic supplementation as well as 4 months after the discontinuation of probiotic treatment.

**Quantification of Cytokines before and after a Lipopolysaccharide (LPS) challenge**

Fold changes of cytokine production upon an LPS challenge were compared before and after 3 months of probiotic supplementation. Quantification of plasma cytokines were performed using human inflammation cytometric bead array kits (BD Biosciences, San Jose, CA, USA) and cytometric bead arrays (Human inflammation kit, BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions to simultaneously detect levels of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12p70, and tumor necrosis factor (TNF)-α.

**Measurement of fecal SCFAs**

The concentrations of SCFAs in fecal samples were analyzed using high-performance liquid chromatography in the National Instrumentation Center for Environmental Management at Seoul National University (Seoul, Korea). Feces were prepared in normal saline (300 µl per 1 g of feces). The fecal slurry supernatants were obtained through centrifugation (2,500 rpm for 10 min). SCFAs, including acetic, butyric, propionic, isovaleric, and valeric acids were measured before and 3 months after probiotic supplementation.

**Microbiome analysis**

*Sample DNA extraction and next generation sequencing (NGS)*
Bacterial genomic DNA was extracted from stool samples using a QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). DNA extraction was performed after homogenization at 30 s for 1 minute using a TissueLyser system (Qiagen) and quantified using a QUBIT 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). For NGS, 16S rRNA gene amplifications and index PCRs were performed following the Illumina 16S metagenomic Sequencing Library preparation guide (Illumina, San Diego, CA, USA). The V3 and V4 regions of 16S rRNA were amplified using the following primer pair (forward 5’–TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG–3’, reverse 5’–TCTCGTGGGCTCGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC–3’). Nextera XT index kits (Illumina, San Diego, California, USA), using 8 cycles, were then used to fragment DNA and add adapter sequences onto the DNA template. Each PCR product was purified using AMPure XP beads (Beckman Coulter, Pasadena, CA, USA). The amplicon library was sequenced by the 2×300 bp paired-end method on a Miseq instrument (Illumina) according to the Illumina protocol.

**Bioinformatics Analysis**

Raw sequencing data were analyzed via QIIME2 (https://docs.qiime2.org/2019.7/) [19]. The fastq files were imported to QIIME2 using the ‘Casava 1.8 paired-end demultiplexed method’ and merged by DADA2 [20]. Filter parameters for trimming and truncating using the DADA2 plugin were 0 and 140 to remove low quality regions of sequences. Feature tables and data generation (‘qiime feature-table summarized’, and ‘qiime feature-table tabulate-seqs’), and phylogenetic tree construction (‘qiime phylogeny-align-to-tree-mafft-fasttree’) were then performed. QIIME 2’s statistical analyses were also performed using the diversity plugin (“core-metrics-phylogenetic”, “alpha-group-significance”, and “beta-group-significance”). To perform taxonomic classification, ‘Greengenes 13_8 99% OTUs full-length sequences’ were used as 16S rRNA gene databases [21, 22].

**Statistical analysis**

All the analyses were performed using SPSS software, version 25.0 (IBM Corporation, USA). Data are expressed as medians [interquartile ranges] according to the distribution. Continuous variables (baseline, after 3 months) were compared using the Wilcoxon signed-rank test. A p-value < 0.05 was considered significant.

**Results**

**Baseline patient characteristics**

Twenty-two patients completed the study. The mean age was 68.1 years and 16 patients (73%) were male. The average time for receiving HD was 8 years and the prevalences of diabetes, hypertension, or histories of gastrointestinal surgeries were 18%, 81.5%, and 22%, respectively (Table 1). Eighty-six percent of patients were taking calcium polystyrene sulfonate and the percentage of patients on various medications, including phosphate binders, antacids, iron, and stool softeners, are reported in Table 2. During the study period, 2 patients received systemic antibiotics: one for community-acquired pneumonia, and the other for calculous cholecystitis.

**The effect of probiotic supplementation on the microbiome and microbial metabolites**

Faith’s phylogenetic diversity showed no significant changes in richness and evenness after probiotic supplementation (p=0.222 and 0.597) (Fig1 A, B). However, at the genus level, the relative abundance of
Bacteroides, Faecalibacterium, Oscillospira, Parabacteroides, Lachnospira, and Akkermansia decreased whereas Bifidobacterium, Ruminococcaceae and Lachnospiraceae increased after probiotic supplementation. (Fig 1C).

**Short chain fatty acids**

SCFAs from gut bacteria are an important nutrient source for enterocytes and are key mediators of the beneficial effects elicited by the gut microbiome. Probiotic supplementation for 3 months resulted in a significant increase of SCFAs such as acetic, butyric, propionic, and valeric acids in feces (Fig. 2).

**Effect of probiotic supplementation on inflammatory responses**

Serum levels of CRP, albumin, calcium, phosphate, and intact parathyroid hormone (iPTH) were similar before and after 3 months of probiotic supplementation. Hemoglobin, total white blood cell and monocyte counts were also comparable before and after probiotic supplementation (Table 3). Despite comparable fecal calprotectin levels (data not shown), serum calprotectin, a marker of acute inflammation, decreased significantly after 3 months of probiotic supplementation (Fig. 3A).

We also compared the cytokine production upon LPS stimulation and found that fold increase of IL-6 after LPS stimulation significantly decreased 3 months after probiotic supplementation (Fig. 3B).

**Effect of probiotic supplementation on CD14^+ CD16^+ pro-inflammatory monocyte subset**

We assessed the impact of probiotic supplementation on circulating pro-inflammatory monocytes. Using flow cytometry, monocyte subpopulations were divided into 3 different subsets: classical, intermediate, and nonclassical monocytes according to CD14 CD16 positivity (Fig. 4A). Although the percent monocytes did not change in complete blood count, the actual number of CD14^+ CD16^+ proinflammatory, non classical monocytes decreased significantly after 3 months of probiotic supplementation (310 vs. 194/mm^2, p < 0.05) (Fig. 4B).

**Effect of probiotic supplementation on regulatory T-cells**

Circulating CD4^+ CD25^+ regulatory T-cells were identified by flow cytometry (Fig. 5A). The percentage of circulating CD4^+ CD25^+ Tregs increased from 3.5% at baseline to 8.6% 3 months after initiating probiotic supplementation (Fig. 5B).

**Tregs and pro-inflammatory monocytes after discontinuation of probiotic supplementation**

The increased percentage of Tregs at 3 months after initiation of probiotic supplementation showed a complete return to baseline levels 4 months after the discontinuation of probiotics (Fig. 6A). The number of pro-inflammatory monocytes that decreased after probiotic supplementation also showed a trend to return to baseline after discontinuation of probiotic supplementation (p = 0.061) (Fig. 6B). However, decreased serum calprotectin level was maintained after discontinuation of probiotics (Fig. 6C).

**Discussion**

In this study, we demonstrated that the probiotic combination of *B. bifidum* BGN4 and *B. longum* BORI had an anti-inflammatory and immunomodulatory effect in patients undergoing maintenance HD. Supplementation of probiotics for 3 months resulted in increased SCFA levels in feces. Serum calprotectin levels as well as IL-6
response upon an LPS challenge decreased significantly 3 months after initiation of probiotic supplementation, and this anti-inflammatory effect was associated with a decreased number of circulating pro-inflammatory, nonclassical monocytes and an increased percentage of immunomodulatory Tregs. However, this effect on circulating immune cells was only transient and returned to baseline after discontinuation of the probiotics.

Emerging evidence shows that intestinal microbiota play an important role in both normal physiology as well as in acute or chronic inflammatory conditions. Both qualitative and quantitative changes in microbiomes have also been reported in uremic animals as well as in patients. Chen et al. reported the increase of relative abundance of Enterobacteriaceae, Ruminococcaceae, and Lachnospiraceae families in CKD patients and Sampaio-Maia et al. showed the decrease of butyrate producing bacteria such as Roseburia, Faecalibacterium, Clostridium, Coprococcus, and Prevotella in end-stage kidney disease (ESKD) (23,24). However, it is not clear whether the perturbation of microbiomes has a causal relationship with CKD. Although a recent meta-analysis of 7 studies enrolling 8-60 dialysis patients showed the potential beneficial effects of probiotics on inflammation, uremic toxins, and GI symptoms, the mechanisms underlying the beneficial effects remains unclear [23].

CKD has emerged as a major cardiovascular risk factor and chronic inflammation is recognized as an important player. The intestine is the single largest organ of the immune system, harboring the largest number of T-cells and macrophages in our body and thus considered to be potent regulator of systemic immunity. Therefore, it can be hypothesized that pre or probiotics induced beneficial effect might be mediated by effects on immune cells. We first observed that probiotic supplementation resulted in decreased numbers of pro-inflammatory monocytes. Monocytes are cells of the innate immune system and have heterogeneous phenotypes according to surface expression of CD14 or CD16 proteins [24]. Among them, CD14+CD16+ are nonclassical monocytes that have been known to invade the endothelium and cause plaque formation in the general population and in patients with CKD [25]. We also demonstrated the expansion of these subsets and also their associations with CRP and vascular stiffness in patients with advanced CKD [26]. Significant decrease of actual number of CD14+CD16+ nonclassical monocytes after probiotic supplementation could suggest that the anti-inflammatory effect of probiotics could be partially mediated by inhibitory effects on the pro-inflammatory monocyte subset. In addition to monocytes, we compared the percentage and number of circulating Tregs before and after probiotic supplementation and observed that the percentage of CD4+CD25+ cells in blood showed a significant increase after 3 months of probiotic supplementation. Mahajan et al., showed that Tregs protect against constant macrophage inflammation and reduce pro-inflammatory cytokine production in murine CKD models [27]. Impaired Treg function was also observed in CKD patients that led to chronic inflammation and subsequent atherosclerosis and cardiovascular diseases [27, 28]. Wang et al., also demonstrated that CKD patients with cognitive dysfunction who had increased percentages of Tregs showed significantly higher mini-mental state examination scores than those with reduced percentages of Tregs [29]. Given that potent immune suppressive function, expansion of circulating Tregs after probiotic supplementation is likely contribute to anti-inflammatory effect in our patients. However, we observed that the increased percentage of Tregs returned to baseline in all patients 4 months after the discontinuation of probiotics. Although statistically insignificant, the number of nonclassical monocytes which decreased after 3 months of probiotics also returned to baseline, showing that the anti-inflammatory, immune modulatory effect of probiotics is only transient.

Unlike other studies, serum CRP level or fecal calprotectin did not show significant difference in our study. Instead, we observed that serum calprotectin levels as well as fold increases of IL-6 after the LPS challenge decreased
significantly after probiotic supplementation. Calprotectin is a heterodimeric complex of 2 S100 calcium-binding proteins: myeloid-related protein (MRP)-8 and MRP-14, which are mainly expressed in neutrophils and monocytes. Although calprotectin has been originally known to be a novel biomarker of disease activity in patients with inflammatory bowel disease when measured in feces, recent data suggests that serum calprotectin levels can be a useful biomarker of disease activity in several acute and chronic inflammatory conditions [30–33]. Serum and urine calprotectin have been demonstrated to be associated with the severity of renal injuries and endothelial dysfunction in Henoch-Schönlein purpura as well as anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis even if CRP levels are not elevated [34–36]. Although we observed that median calprotectin levels decreased significantly after 3 months of probiotic supplementation, the usefulness of serum calprotectin levels as a sensitive marker of inflammation in patients with CKD or HD requires further examination.

IL-6 has been demonstrated to be an important player in the progression of CKD[37]. It exacerbates inflammation as well as endothelial injury by reducing endothelial nitric oxide synthase (eNOS) and injection of recombinant IL-6 exacerbates atherosclerosis in mice [38]. Our data showing that the fold increase of IL-6 upon LPS challenge significantly decreased after 3 months of probiotic supplementation also supports its anti-inflammatory effect.

Various metabolites produced by intestinal microbiota are important in both normal physiology and in pathological conditions. SCFAs are known to be important in colonocyte survival, maintaining barrier integrity and immune modulation. In contrast, excessive production of trimethylamine (TMA) due to the consumption of an animal protein-rich diet has been shown to be associated with atherosclerosis and cardiovascular diseases. In our study, we observed that the levels of acetic, butyric, propionic, and valeric acids increased significantly after 3 months probiotic supplementation. SCFAs are bacterial fermentation products with pleiotropic functions including lipid regulation, energy metabolism, and immune regulation via interaction with G protein-coupled receptors, histone deacetylases, or direct humoral effects. Given that SCFAs have immunomodulatory effects, it is possible that significantly elevated levels of SCFAs during the probiotic supplementation might be partially responsible for decreased proinflammatory monocytes and expansion of Tregs.

Even though our data showed that probiotics elicited a very potent anti-inflammatory effect, 2 of the patients in our study required hospitalization. One patient was diagnosed with calculous cholecystitis, underwent a laparoscopic cholecystectomy, and was administered systemic antibiotics. The second patient was diagnosed with community-acquired pneumonia 1 month of probiotic supplementation and was discharged after 5 days of systemic antibiotic therapy. Although a causal relationship between probiotic use and serious infections in this study was not clear, one should always be aware that excessive suppression of inflammatory responses might increase the risk of infections.

**Conclusion**

Probiotic combinations of *B. bifidum* BGN4 and *B. longum* BORI reduced systemic inflammatory responses and this effect was partially mediated by an increase of Tregs and a decrease of the nonclassical pro-inflammatory monocyte subset. Larger-scale clinical studies testing the components of microbiota or their metabolites and assessing the effects on long-term outcomes in dialysis patients are needed.

**Abbreviations**

HD : hemodialysis
Tregs: regulatory T-cells
CKD: chronic kidney disease
CFU: colony forming units
PCR: polymerase chain reaction
FDA: food and drug administration
NGS: next generation sequencing
SCFA: short chain fatty acids
iPTH: intact parathyroid hormone
ESKD: end stage kidney disease
ANCA: anti-neutrophil cytoplasmic antibody
CRP: C-reactive protein
eNOS: endothelial nitric oxide synthase

Declarations

Funding
This study was supported by the Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education (2017R1A2B1002734)

Conflict of interest
The researcher claims no conflicts of interest.

Ethics approval
The study protocol was approved by the Korea University Medical Center Institutional Review Board (IRB No. 2018AN0346).

Consent to participate
Written informed consent was provided by all participants.

Consent for publication
Consent for publication was obtained from all authors

Availability of data and material (data transparency)
All data support published claims and comply with field standards.
Authors' contribution

Eunho Choi, Ji hyun Yang, Sang Kyung Jo: Conception, design, analysis and interpretation of data, Drafting and revising the article, final approval of the version to be published

Myeong Soo Park, Yeoungje Seoung: analysis and interpretation of data, revising the article, final approval of the version to be published

Geun-Eog Ji, Se Won Oh, Myung Gyu Kim, Won Young Cho: providing intellectual content and final approval of the version to be published

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Tables

Table 1. Patient demographic characteristics
<table>
<thead>
<tr>
<th>Age</th>
<th>68.1 (40-84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>16 (73%)</td>
</tr>
<tr>
<td>Women</td>
<td>6 (27%)</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>22.76 (17.6-27.5)</td>
</tr>
<tr>
<td>Hemodialysis year</td>
<td>8 (1994-2018)</td>
</tr>
<tr>
<td>Peritoneal dialysis history</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>Kidney transplantation history</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (81%)</td>
</tr>
<tr>
<td>Heart failure</td>
<td>10 (45%)</td>
</tr>
<tr>
<td>Ischemic heart disease</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>Gastrointestinal operation history</td>
<td>5 (22%)</td>
</tr>
<tr>
<td>Cancer</td>
<td>5 (22%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Liver disease</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Chronic obstructive pulmonary disease</td>
<td>2 (9%)</td>
</tr>
</tbody>
</table>

**Table 2. Medications**

<table>
<thead>
<tr>
<th>Medication</th>
<th>Count (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium binder</td>
<td>19 (86%)</td>
</tr>
<tr>
<td>Statin</td>
<td>14 (63%)</td>
</tr>
<tr>
<td>Phosphate binder</td>
<td>19 (86%)</td>
</tr>
<tr>
<td>Ca containing</td>
<td>9 (40%)</td>
</tr>
<tr>
<td>Non-Ca containing</td>
<td>10 (46%)</td>
</tr>
<tr>
<td>Oral iron</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>4 (18%)</td>
</tr>
<tr>
<td>Antacid</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Steroid</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Stool softener</td>
<td>2 (9%)</td>
</tr>
<tr>
<td>Antihistamine</td>
<td>1 (4%)</td>
</tr>
</tbody>
</table>

**Table 3. Clinical parameters; baseline and post probiotics supplement**
<table>
<thead>
<tr>
<th></th>
<th>0 Month (n=22)</th>
<th>3 Month (n=18)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>10.5 [9.9,11.0]</td>
<td>10.4 [9.9,11.6]</td>
<td>0.563</td>
</tr>
<tr>
<td>Platelet (x1000/uL)</td>
<td>176.5 [139.8,192.0]</td>
<td>153.5 [117.0,198.2]</td>
<td>0.765</td>
</tr>
<tr>
<td>WBC (x1000/uL)</td>
<td>5900 [5050,7730]</td>
<td>5739±2056</td>
<td>0.247</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>60.6 [56.6,64.1]</td>
<td>60.9 [55.3,65.5]</td>
<td>0.751</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>23.5 [20.6,26.7]</td>
<td>23.2 [20.2,27.1]</td>
<td>0.968</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>9.6 [7.9,11.3]</td>
<td>9.0 [7.9,11.6]</td>
<td>0.588</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>4.2 [3.2,6.2]</td>
<td>4.8 [2.7,7.9]</td>
<td>0.490</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>0.7 [0.5,1.0]</td>
<td>0.7 [0.5,1.0]</td>
<td>0.370</td>
</tr>
<tr>
<td>CRP-hs (mg/L)</td>
<td>3.8±6.8</td>
<td>1.0 [0.6,7.7]</td>
<td>0.370</td>
</tr>
<tr>
<td>BUN Pre-HD (mg/dL)</td>
<td>57.9 [41.1,73.0]</td>
<td>64.8 [47.8,71.5]</td>
<td>1.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>8.8 [7.5,10.0]</td>
<td>8.5 [7.3,11.7]</td>
<td>0.526</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>138.0 [137.7,139.2]</td>
<td>139.0 [135.0,140.0]</td>
<td>0.775</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>4.5 [4.1,5.4]</td>
<td>5.7 [4.1,5.0]</td>
<td>0.245</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>101.0 [98.0,104.0]</td>
<td>100.5 [98.0,102.5]</td>
<td>0.506</td>
</tr>
<tr>
<td>CO2, total (mmol/L)</td>
<td>22.0 [20.8,24.0]</td>
<td>22.5 [19.0,23.3]</td>
<td>0.773</td>
</tr>
<tr>
<td>Ca, total (mg/dL)</td>
<td>9.0 [8.3,9.5]</td>
<td>13.3±19.1</td>
<td>0.519</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>4.8 [3.4,6.1]</td>
<td>5.0 [3.9,6.3]</td>
<td>0.466</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>1.1±0.1</td>
<td>1.1±0.1</td>
<td>0.799</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.3 [3.7,5.2]</td>
<td>5.1±1.0</td>
<td>0.104</td>
</tr>
<tr>
<td>Protein (g/dL)</td>
<td>6.7 [6.5,7.0]</td>
<td>6.8 [6.4,7.1]</td>
<td>0.773</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.0 [3.8,4.1]</td>
<td>3.9 [3.7,4.3]</td>
<td>0.095</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>115.0 [101.3,129.8]</td>
<td>120.0 [94.3,154.8]</td>
<td>0.360</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>19.0 [14.5,22.8]</td>
<td>20.0 [13.0,25.3]</td>
<td>0.760</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>14.5 [11.0,18.0]</td>
<td>14.0 [12.0,18.3]</td>
<td>0.647</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>92.5 [69.5,118.3]</td>
<td>87.5 [70.3,106.5]</td>
<td>0.177</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>20.5 [12.5,28.5]</td>
<td>20.5 [13.0,29.0]</td>
<td>0.061</td>
</tr>
<tr>
<td>Bilirubin, total (mg/dL)</td>
<td>0.5 [0.4,0.7]</td>
<td>0.5 [0.5,0.8]</td>
<td>0.268</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>97.0 [84.0,108.0]</td>
<td>96.5 [90.8,109.8]</td>
<td>0.525</td>
</tr>
<tr>
<td>KT/V</td>
<td>1.67 ± 0.24</td>
<td>1.72 ± 0.25</td>
<td>0.223</td>
</tr>
<tr>
<td>URR</td>
<td>75.07±5.27</td>
<td>76.2 ± 4.71</td>
<td>0.091</td>
</tr>
<tr>
<td>Body weight</td>
<td>61.6 ± 11.4</td>
<td>61.2 ± 11.4</td>
<td>0.808</td>
</tr>
</tbody>
</table>
Figures

Figure 1.

(A) Faith's PD

(B) Pielou's Evenness

(C) Microbiome composition alteration at the genus level

Figure 1

Microbiome analysis. (A) Richness using Faith's Phylogenetic Diversity (PD). (B) Community's Pielou's evenness. (C) Microbiome composition alteration at the genus level.
Figure 2

Effect of probiotic supplementation on fecal short chain fatty acids level. *p<0.05 compared to baseline.

Figure 3

(A) Serum Calprotectin

(B) IL-12 p70, TNF-α, IL-10, IL-6, IL-1β, IL-8
Effect of probiotic supplementation on inflammatory markers. (A) Serum calprotectin level. (B) Fold increase of cytokines after LPS stimulation. *p<0.05 compared to baseline.

**Figure 4.**

Effect of probiotic supplementation on proinflammatory, non classical monocyte subset. (A) Identification of monocyte subset according to CD14 and CD16 by flow cytometry. (B) Number of CD14+ CD16+ nonclassical monocytes. *p<0.05 compared to baseline.

**Figure 6.**

Effect of discontinuation of probiotics on immune cells and calprotectin level. (A) Percentage of CD4+ CD25+ regulatory T cells. (B) Number of CD14+ CD16+ nonclassical monocytes. (C) Serum calprotectin level. *p<0.05 compared to baseline.